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[Title of Invention] Biosensor

[Abstract]
[Problem]
A biosensor is needed which can measure the concentration of a substrate such as glucose without being affected by the presence of oxygen dissolved in the sample liquid.

[Means of Solution]
The biosensor of the present invention comprises an electrically insulating base plate, an electrode system having a working electrode and a counter electrode formed on said base plate, and a reaction layer formed on said electrode system, wherein said reaction layer contains a dehydrogenase and electron acceptor, said dehydrogenase being combined with pyrroloquinoline quinone as coenzyme.

[Claims]
1. A biosensor comprising an electrically insulating base plate, an electrode system having a working electrode and a counter electrode formed on said base plate, and a reaction layer formed on said electrode system, said sensor being characterized by said reaction layer that contains dehydrogenase and electron acceptor, said dehydrogenase being combined with pyrroloquinoline quinone as a coenzyme.

2. The biosensor according to claim 1, wherein said reaction layer further contains pyrroloquinoline quinone.

3. The biosensor according to claim 2, wherein the content of said pyrroloquinoline quinone is from 1.5×10^{-6} to 1.5×10^{-4} grams per square centimeter of the reaction layer.

4. The biosensor according to claim 1 or 2, wherein a dehydrogenase, which is combined with said pyrroloquinoline quinone as coenzyme, is glucose dehydrogenase or fructose dehydrogenase.

5. The biosensor according to claim 4, wherein the quantity of said glucose dehydrogenase is from 1 unit to 200 units per square centimeter of the reaction layer.

[Detailed Description of the Invention]
[0001]
[Technical Field of the Invention]
This invention relates to a biosensor which can quantitatively measure a specified ingredient in a living-body sample such as blood or urine; or a raw material or product in the food industry including fruit juice quickly, easily, and with a high degree of precision.

[0002]
[Prior Art]
Biosensors which can quantitatively measure a specified ingredient in living-body samples or food without diluting or stirring the sample liquid have been proposed. For example, in the official publication of applied patent Heisei 3 (1991) 202764, a biosensor has been

disclosed in which an electrode system is formed on an insulating base plate by screen printing or other method and a reaction layer including an oxidoreductase and electron acceptor. This biosensor quantitatively measures the concentration of a specified ingredient in the sample as follows: First, the reaction layer is dissolved by dropping the sample onto the reaction layer, and an enzyme reaction proceeds between the specified ingredient in the sample liquid and the oxidoreductases in the reaction layer. This enzyme reaction reduces the electron acceptor. After a short interval, a voltage applied across the sensor electrodes electrochemically oxidizes the reduced electron acceptor. The concentration of the specified ingredient in the sample liquid is obtained quantitatively from the oxidation current of this reaction.

[0003]
[Problems to be Solved by the Invention]
It is known that among such biosensors, glucose oxidase as an oxidoreductase is commonly used to detect glucose. When glucose oxidase is used, however, the electron acceptor is reduced as part of the enzyme reaction, as is the enzyme dissolved in the sample liquid. Although the reduced electron acceptor can be readily oxidized electrochemically, hydrogen peroxide, a reduced byproduct of dissolved oxygen, cannot. Therefore, the value of the oxidation current obtained by applying a voltage across the sensor electrodes corresponds to the degree of oxidation of the reduced electron acceptor, not including the degree of oxidation of the hydrogen peroxide. The original concentration of the specified ingredient, accordingly, cannot be measured accurately using the obtained value of the oxidation current. To gain an accurate figure for the concentration of the specified ingredient, a preparatory process such as prior quantitative measurement of dissolved oxygen in the sample liquid was needed. This invention aims to offer a biosensor which can precisely measure the concentration of a specified ingredient such as glucose without being influenced by the presence of oxygen dissolved in the sample liquid.

[0004]
[Means of Solving the Problem]
To solve the above-mentioned problem, the biosensor of this invention comprises an electrically insulating base plate, an electrode system having a working electrode and a counter electrode formed on said base plate, and a reaction layer formed on said electrode system, wherein said reaction layer contains dehydrogenase and an electron acceptor, said dehydrogenase being combined with pyrroloquinoline quinone as a coenzyme.

[0005]
[Embodiment of the Invention]
The biosensor of the invention is characterized by the reaction layer of the sensor containing dehydrogenase combined with pyrroloquinoline quinone as a coenzyme. The dehydrogenase that is combined with pyrroloquinoline quinone as a coenzyme reduces only the electron acceptor when the enzyme acts on the specified ingredient. Accordingly, the concentration of the specified ingredient can be measured accurately from the value of the oxidization current. As examples of dehydrogenase combined with pyrroloquinoline quinone as a coenzyme, glucose dehydrogenase and fructose dehydrogenase are included. Their optimal concentration is 1 to 200 units per square centimeter of the reaction layer; more preferably 4 to 100 units. One unit means the amount of oxidoreductase able to oxidize of 1 micro mole of the specified ingredient in one minute. If the content of glucose dehydrogenase is less than 1 unit per square centimeter of reaction layer, the above-mentioned oxidization time is prolonged to several minutes. If the amount of glucose dehydrogenase exceeds 200 units per square centimeter of the reaction layer, the production costs rise and the response current tends to disperse because the reaction layer cracks easily during manufacture.

[0006]
The reaction layer of this invention should ideally contain pyrroloquinoline quinone in addition to above-mentioned dehydrogenase, since the detection sensitivity improves and the concentration of the specified ingredient can be detected over a wider range. The sodium salt of pyrroloquinoline quinone can be used as the pyrroloquinoline quinone. The amount of pyrroloquinoline quinone is ideally 1.5×10^{-6} to 1.5×10^{-4} grams per square centimeter of the reaction layer; more preferably 1.0×10^{-5} to 8.0×10^{-5} grams. Furthermore, the reaction layer of this invention contains an electron acceptor. As examples of electron acceptors, ferricyanide ion, p-benzoquinone and its derivatives, phenazine methosulfate, methylene blue, and ferrocene and its derivatives are included. One or more of these are used as the electron acceptor. Ferricyanide ion shows the best performance.

[0007]
The reaction layer of the biosensor used in this invention may contain hydrophilic macromolecules in addition to the above-mentioned enzyme and electron acceptor. By adding a hydrophilic macromolecule to the reaction layer, detachment of the reaction layer from the surface of the electrode system can be prevented. Furthermore, cracking of the reaction layer is prevented, contributing overall to improved reliability of the biosensor. As examples of hydrophilic macromolecules, carboxymethyl cellulose, hydroxyethyl cellulose, hydroxypropyl cellulose, methyl cellulose, ethyl cellulose, ethyl hydroxyethyl cellulose, carboxymethylethyl cellulose, polyvinylpyrrolidone, polyvinyl alcohol, polyamino acids such as polylysine, polystyrene sulfonic acid, gelatin and its derivatives, acrylic acid and its salts, metaacrylic acid and its salts, starch and its derivatives, and anhydrous maleic acid and its salts are included. Carboxymethyl cellulose shows the best performance. There are two methods for the measurement of oxidization current: the two-electrode method, in which only a working electrode and a counter electrode are used; and the more accurate three-electrode method, in which a reference electrode is added.

[0008]
[Embodiment Examples]
The invention will be hereafter described precisely using specific examples of embodiments. Figure 1 is the outline ground plan of the biosensor of this invention without the reaction layer. Leads 2 and 3 are formed on a base plate 1 made of polyethylene terephthalate by printing silver paste using screen printing. Working electrode 4 is then formed on base plate 1 by printing electroconductive carbon paste containing a resin binder. This working electrode makes electrical contact with lead 2. Furthermore, an insulating layer 6 is formed on the base plate 1 by printing insulating paste. Insulating layer 6 covers the peripheral part of working electrode 4, keeping the area of exposed part of working electrode 4 constant. A ring-shaped counter electrode 5 is formed on base plate 1 by printing electroconductive carbon paste containing resin binder so as to make electrical contact with lead 3. A synthetic resin board of such as polyethylene terephthalate is used as the insulating base plate. For the above-mentioned leads and electrodes, platinum, gold, palladium, etc. may also be used in addition to silver and carbon. Figure 2 shows a longitudinal section of a biosensor of this invention. As shown in Fig. 1, a reaction layer 7 containing enzymes and an electron acceptor is formed on insulating base plate 1 on which the electrode system is formed.

[0009]
[Embodiment Example 1]
On the electrode system of the base plate in Fig. 1, reaction layer 7 was formed by dropping a liquid mixture of glucose dehydrogenase (hereafter abbreviated to GDH) and potassium ferricyanide onto the base plate. The quantity of GDH contained in reaction layer 7 was 30 units per square centimeter of the reaction layer. Next, an aqueous solution of glucose was dropped onto reaction layer 7. When a sample liquid containing glucose is supplied to the

reaction layer, glucose in the sample is oxidized by the glucose dehydrogenase. At the same time, the electron acceptor in the reaction layer is reduced. One minute after dropping the liquid, a voltage of +0.5 V was applied to the working electrode 4 with reference to the counter electrode. The current was measured five seconds later. Because this current is proportional to the concentration of the generated reduced electron acceptor, that is, to the concentration of the specified ingredient in the sample liquid, the glucose concentration in the sample can be obtained by measuring the current. The samples were divided into two groups: the dissolved oxygen concentration was about 30 mmHg in one group and about 180 mmHg in the other. The glucose concentration in the sample was varied in each group. The results demonstrated a correlation between response current and glucose concentration. The linearity was fairly good regardless of the concentration of dissolved oxygen.

[0010]
[Example for Comparison 1]
Reaction layer 7 was formed in the same manner as in Embodiment Example 1 with the exception that glucose oxidase was used instead of glucose dehydrogenase. The correlation between the response current and glucose concentration was examined using the same samples as in Embodiment Example 1. The results revealed that the obtained response current varied according to the dissolved oxygen concentration with a slight tendency for a higher dissolved oxygen concentration to result in a smaller response current.

[0011]
[Embodiment Example 2]
An aqueous solution containing GDH, potassium ferricyanide, and pyrroloquinoline quinone (hereafter abbreviated to PQQ) was dropped onto the base plate shown in Fig. 2. Reaction layer 7 was then formed by drying the solution. The amount of GDH and PQQ contained in reaction layer 7 was 20 units and 4.5×10^{-5} grams per square centimeter of the reaction layer, respectively. The correlation between response current and glucose concentration was also examined using the same samples as in Embodiment Example 1. A much higher degree of correlation than in Embodiment Example 1 was obtained between response current and glucose concentration.

[0012]
[Embodiment Example 3]
An aqueous solution containing fructose dehydrogenase (hereafter abbreviated to FDH) and potassium ferricyanide was dropped onto the base plate 1 shown in Fig. 2. Reaction layer 7 was then formed by drying the solution. The correlation between response current and glucose concentration was also examined using the same samples as in Embodiment Example 1. A high correlation was obtained between response current and glucose concentration. In addition, this result was not influenced by the concentration of dissolved oxygen in the sample. Furthermore, it was recognized that the response improved when PQQ was added to the reaction layer.

[0013]
[Effect of the Invention]
According to this invention, a new biosensor can be obtained by which a specified ingredient contained in a living-body sample such as blood or urine; or samples of materials or products in the food industry can be measured accurately and rapidly.

[Brief Explanation of Drawings]
[Figure 1]
Figure 1 is an outline ground plan of an embodiment of the invention with the reaction layer omitted.

[Figure 2]
Figure 2 is a longitudinal section of the main part of the biosensor.

[Explanation of Marked Parts]
1 Electrically insulating base plate
2 and 3 Leads
4 Working electrode
5 Counter electrode
6 Insulating layer
7 Reaction layer

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FIG. 1

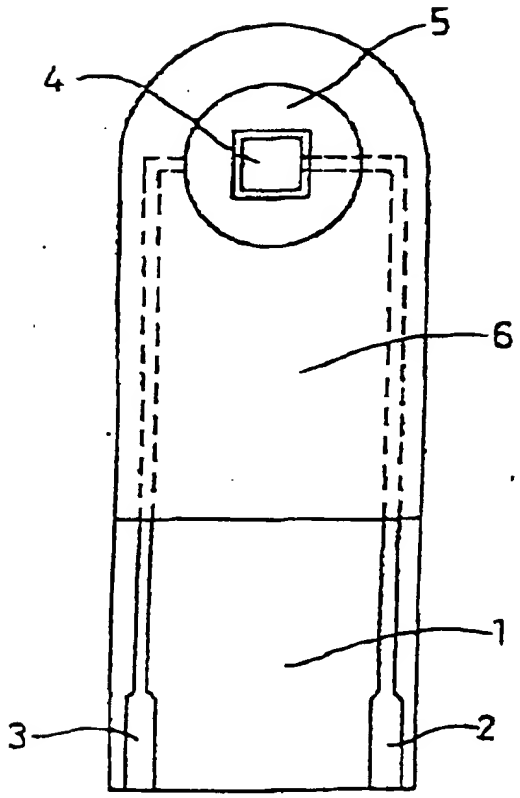

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FIG. 2